RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL ARTICLE

# Expert consensus on dynamics of laboratory tests for diagnosis of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis

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**To cite:** Ravelli A, Minoia F, Davì S, *et al*. Expert consensus on dynamics of laboratory tests for diagnosis of macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *RMD Open* 2015;**1**: e000161. doi:10.1136/rmdopen-2015-000161

► Prepublication history for this paper is available online. To view these files please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2015-000161).

Received 4 August 2015
Revised 8 September 2015
Accepted 12 September 2015

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## ABSTRACT

**Objective:** To identify which laboratory tests that change over time are most valuable for the timely diagnosis of macrophage activation syndrome (MAS) complicating systemic juvenile idiopathic arthritis (sJIA).

**Methods:** A multistep process, based on a combination of expert consensus and analysis of real patient data, was conducted. A panel of experts was first asked to evaluate 115 profiles of patients with MAS, which included the values of laboratory tests at the pre-MAS visit and at MAS onset, and the change in values between the two time points. The experts were asked to choose the 5 laboratory tests in which change was most important for the diagnosis of MAS and to rank the 5 selected tests in order of importance. The relevance of change in laboratory parameters was further discussed and ranked by the same experts at a consensus conference.

**Results:** Platelet count was the most frequently selected test, followed by ferritin level, aspartate aminotransferase (AST), white cell count, neutrophil count, and fibrinogen and erythrocyte sedimentation rate. Ferritin was most frequently assigned the highest score. At the end of the process, platelet count, ferritin level and AST were the laboratory tests in which the experts found change over time to be most important.

**Conclusions:** We identified the laboratory tests in which change over time is most valuable for the early diagnosis of MAS in sJIA. The dynamics of laboratory values during the course of MAS should be further scrutinised in a prospective study in order to establish the optimal cut-off values for their variation.

## Key messages

**What is already known on this subject?**

- The change in laboratory values over time may be more relevant for making an early diagnosis of macrophage activation syndrome (MAS) in the setting of systemic juvenile idiopathic arthritis (sJIA) than the achievement of the absolute threshold required by current diagnostic criteria.

**What might this study add?**

- The laboratory tests in which changes over time are most valuable for the timely diagnosis of MAS occurring in the context of sJIA were identified through a data-driven and consensus formation approach.

**How might this impact on clinical practice?**

- Platelet count, serum ferritin and aspartate aminotransferase level are the laboratory biomarkers in which changes over time are most helpful for the early detection of MAS in patients with sJIA.

## INTRODUCTION

Macrophage activation syndrome (MAS) is a hyperinflammatory complication of systemic juvenile idiopathic arthritis (sJIA) caused by a highly stimulated but dysregulated immune response that involves the sustained activation and expansion of T lymphocytes and macrophages, and results in a cytokine storm syndrome.[1–4] It is a serious and potentially fatal condition, responsible for much of the mortality observed in sJIA.[5 6] MAS complicates at least 10% of cases of sJIA, but a much higher proportion of patients (30–40%) show signs of subclinical MAS.[7 8]

Because MAS can pursue a rapidly fatal course if left untreated, it requires prompt recognition to initiate appropriate treatment and prevent deleterious outcomes. However, early diagnosis is frequently difficult, given the lack of a single pathognomonic clinical or laboratory parameter. Furthermore, histopathological haemophagocytosis may not be detected in the initial stages,[9 10] or might not be discovered at all, and lacks specificity for haemophagocytic syndromes.[11] In addition, the features of MAS may be hard to distinguish from those conditions presenting with overlapping manifestations, such as flares of sJIA or systemic infections. The diagnostic challenges are compounded by the variability in the frequency and severity of the typical clinical and laboratory features of the syndrome across patients.[12 13]

The difficulties in making the diagnosis highlight the need for accurate criteria to aid physicians in identifying MAS in its earliest stages and in distinguishing it from other conditions. Historically, two sets of guidelines have been proposed for diagnosis of MAS in the setting of sJIA: the diagnostic guidelines for haemophagocytic lymphohistiocytosis (HLH)-2004[14] and the preliminary diagnostic guidelines for MAS complicating sJIA.[15] A set of classification criteria for sJIA-associated MAS was recently developed through a multinational collaborative effort.[16]

Although all these criteria are considered suitable for detecting MAS in sJIA, it has been argued that the relative change in laboratory values over time may be more relevant for making an early diagnosis than the decrease below, or increase above, a certain threshold, as stipulated by the criteria.[1 16–19] Note that patients with active sJIA often have elevated platelet counts as well as increased levels of ferritin or fibrinogen as part of the underlying inflammatory process.[20 21] Thus, the occurrence of a relative decline (in the case of platelet count or fibrinogen) or elevation (in the case of ferritin) in these laboratory biomarkers, rather than the achievement of an absolute threshold required by the criteria, may be sufficient to herald the occurrence of MAS in the setting of sJIA.[12 18]

One of the objectives of the international collaborative project that led to the development of the novel classification criteria for MAS complicating sJIA,[16] was to identify the laboratory tests in which change over time is most valuable for the timely diagnosis of MAS occurring in the context of sJIA. The results of this effort are described in the present paper.

## METHODS

### Study design and data collection procedure

The multistep process strategy used in developing the classification criteria for MAS complicating sJIA has been described in detail elsewhere.[12 13 22] Briefly, in the first phase of the project, international paediatric rheumatologists and paediatric haematologists were asked to participate in a retrospective cohort study of patients with sJIA-associated MAS and with two conditions potentially confusable with MAS, represented by active sJIA not complicated by MAS, and systemic infection. A total of 1111 patients, 362 with sJIA-associated MAS, 404 with active sJIA without MAS and 345 with systemic infection, were reported by 95 paediatric subspecialists practising in 33 countries on five continents. The features of these patients have been described elsewhere.[12 13 22] For the purposes of the present study, only data of patients with MAS were evaluated.

Collected information included laboratory features at three time points: (1) at last visit before onset of MAS; (2) at onset of MAS (defined as the time when the initial clinical and/or laboratory abnormalities suggesting the occurrence of MAS were detected) and (3) at full-blown MAS (defined as the time at which MAS reached its most severe stage). Because the present study aimed to scrutinise the performance of the change in laboratory tests in identifying MAS in its earlier stages, only laboratory values recorded at last visit before onset of MAS and at MAS onset were retained, and the change in values was calculated between these two time points. All laboratory parameters were tested using the original values provided by each local laboratory.

### Web-based consensus procedures among the experts

The second step of the process consisted of the evaluation and ranking of the change over time in the most typical laboratory parameters of MAS by a panel of experts. The expert panel included 20 paediatric rheumatologists and 8 paediatric haematologists, selected on the basis of their publication records and experience in the care of children with MAS and related disorders.

The experts were asked to evaluate a total of 115 profiles of patients with sJIA with MAS. These profiles were selected randomly among the 362 patients with sJIA with MAS according to their caring physician. However, preference was given to the patients who had data for at least five laboratory parameters at both aforementioned time points available. A bias in the selection of patients was unlikely, as the characteristics of selected and unselected patients were comparable (data not shown). Each patient profile included the values of laboratory parameters at last visit before onset of MAS and at onset of

MAS, the normal range of each parameter at the local laboratory, and the absolute and percentage change of values between the two time points. The following 11 laboratory tests were assessed: white cell count (WCC), neutrophil count, haemoglobin, platelet count, erythrocyte sedimentation rate (ESR), aspartate aminotransferase (AST), lactic dehydrogenase, fibrinogen, triglycerides, ferritin and D-dimer.

Based on these data, all the experts were first asked to classify each patient profile as MAS or non-MAS, that is, to confirm or not to confirm the diagnosis of MAS made by the caring physician. If the diagnosis of MAS was confirmed, the expert was first asked to select the five laboratory tests in which change over time was most important in influencing his or her decision to categorise the patient as having MAS. Then, the expert was asked to rank the five selected laboratory tests in order of importance by assigning 5 to the most important and 1 to the least important test.

The minimum level of agreement among the experts about patient classification as MAS or non-MAS was set at 80%. If an 80% consensus was not attained, the patient profile was discussed in a further round. Two rounds of voting were used, with comments and voting from participants available, to augment the number of consensus decisions. Profiles for which consensus was not achieved at the final round were declared non-interpretable and discarded from further analyses. Profiles for which consensus was reached among the experts about the diagnosis of non-MAS were also discarded.

All web-based consensus procedures were performed electronically and conducted by the Pediatric Rheumatology International Trials Organization (PRINTO).

### Ranking of laboratory tests at consensus conference

The International Consensus Conference on MAS Classification Criteria was held in Genoa, Italy, on 21–22 March 2014. The meeting was attended by all 28 experts who participated in the web-consensus evaluations and was facilitated by two moderators (NR and HB) with expertise in nominal group technique (NGT). The first day of the conference was devoted to the development of the classification criteria for MAS complicating sJIA,[16] whereas the diagnostic role of change in laboratory parameters was addressed on the second day.

Before the start of consensus evaluations, a plenary session was held to illustrate the scope, methodology and flow of the overall project, the results of web-based consensus procedures and the methodology of the NGT. For the specific purpose of the present project, participants were shown the results of the web-consensus evaluation of the relative diagnostic importance of the change over time in the laboratory tests. These results included the percentage of instances in which each test was selected by the experts as well as the frequency of individual scores (from 1 to 5) and the sum of scores assigned to each test by the experts.

Participants were then randomised into two equally sized nominal groups and, using NGT, were first asked to electronically select, independently of each other, the five laboratory parameters in which change over time was felt most important in the early diagnosis of MAS, and then to rank the five selected parameters assigning 5 to the most important and 1 to the least important. Voters were advised to base their choices on their opinion about which of the laboratory tests and respective changes were easiest to use, and most credible (face/content validity). The experts were connected by laptops to a central computer and submitted all their rankings electronically.

## RESULTS

### Results of the web-based consensus procedures among the experts

After two rounds of web evaluations, the experts achieved consensus on the classification of 103 (89.6%) of the 115 patient profiles examined. Seventy patients (60.9%) were classified as MAS, whereas 33 patients (28.7%) were classified as non-MAS. For 12 patients (10.4%), consensus was not reached. The 45 profiles classified as non-MAS or for which consensus among the experts was not reached, were discarded. Table 1 shows the comparison of demographic, clinical and histopathological features, triggers, therapeutic interventions and outcome between patients classified as MAS or non-MAS by the expert panel. Compared with patients classified as non-MAS, patients who had the diagnosis of MAS confirmed by the experts were younger at onset of MAS, and had a greater frequency of fever and of most of the other typical clinical features of the syndrome, had more frequently undergone a bone marrow aspiration or a lymphnode or liver biopsy, were admitted more commonly to the intensive care unit and had a greater frequency of death. The gender ratio, duration of sJIA at MAS onset, triggers and therapeutic interventions as well as the frequency of bone marrow or biopsy haemophagocytosis, were comparable between the two groups. The comparison of the change in laboratory parameters over time between patients diagnosed as MAS or non-MAS by the experts is presented in table 2. Overall, patients who had the diagnosis of MAS confirmed by the experts had a greater change in laboratory values than those classified as non-MAS.

Table 3 shows, for each laboratory test evaluated by the experts in the 70 patient profiles for which consensus about the diagnosis of MAS was achieved, the number and percentage of instances in which the test was selected and the number of instances in which each individual score was assigned to the test. The platelet count was the most frequently selected test and achieved the highest global score, followed by ferritin, AST, WCC, neutrophils, fibrinogen and ESR. However, ferritin was

**Table 1** Comparison of demographic, clinical and histopathological features, triggers, treatments and outcome between patients classified as MAS or non-MAS, by the expert panel*

| | N | Patients classified as MAS (n=70) | N | Patients classified as non-MAS (n=33) | p Value |
|---|---|---|---|---|---|
| *Demographic characteristics* | | | | | |
| Sex | 70 | | 33 | | 0.81 |
| Female | | 42 (60.0) | | 19 (57.6) | |
| Male | | 28 (40.0) | | 14 (42.4) | |
| Age at onset of MAS, median (IQR), years | 69 | 10.5 (4.4–14.6) | 32 | 7.6 (4.1–11.6) | 0.01 |
| Duration of systemic JIA at MAS onset, median (IQR), years | 69 | 0.9 (0.1–2.4) | 31 | 1.5 (0.1–4.1) | 0.31 |
| *Clinical manifestations at onset of MAS* | | | | | |
| Fever | 69 | 68 (98.5) | 33 | 28 (84.9) | 0.01† |
| Hepatomegaly | 67 | 50 (74.6) | 33 | 16 (48.5) | 0.01 |
| Splenomegaly | 65 | 37 (56.9) | 33 | 16 (48.5) | 0.43 |
| Lymphadenopathy | 65 | 36 (55.4) | 32 | 8 (25.0) | 0.005 |
| Active arthritis | 69 | 42 (60.9) | 33 | 17 (51.5) | 0.37 |
| Central nervous system involvement | 65 | 31 (47.7) | 33 | 8 (24.2) | 0.02 |
| Haemorrhagic manifestations | 67 | 26 (38.8) | 33 | 4 (12.1) | 0.006 |
| Heart, lung or kidney failure | 69 | 13 (18.8) | 33 | 1 (3.0) | 0.03 |
| *Triggers* | | | | | 0.49‡ |
| Active disease | 58 | 28 (48.3) | 27 | 14 (51.8) | – |
| Infection | 58 | 23 (39.6) | 27 | 7 (25.9) | – |
| Treatment toxicity | 58 | 1 (1.7) | 27 | 3 (11.1) | – |
| Other | 58 | 3 (5.2) | 27 | 2 (7.4) | – |
| Unknown | 58 | 3 (5.2) | 27 | 2 (7.4) | – |
| *Histopathological features* | | | | | |
| Bone marrow aspiration and/or biopsy of l ymphnode and/or liver | 69 | 54 (78.3) | 33 | 16 (48.5) | 0.002 |
| Haemophagocytosis on bone marrow aspiration and/or biopsy of lymphnode and/or liver | 54 | 31 (57.4) | 16 | 11 (68.7) | 0.42 |
| *Therapeutic interventions* | | | | | |
| Any corticosteroids | 69 | 68 (98.5) | 33 | 33 (100.0) | 1.0 |
| Cyclosporine | 69 | 47 (68.1) | 33 | 24 (72.7) | 0.64 |
| Intravenous immunoglobulin | 68 | 27 (39.7) | 33 | 10 (30.3) | 0.36 |
| Biological medications† | 68 | 17 (25.0) | 33 | 9 (27.3) | 0.81 |
| Etoposide | 67 | 10 (14.9) | 33 | 4 (12.1) | 1.0 |
| Other immunosuppressants | 66 | 5 (7.6) | 32 | 3 (9.1) | 0.71 |
| Plasma exchange | 67 | 6 (9.0) | 33 | 2 (6.1) | 1.0 |
| *Outcome* | | | | | |
| ICU admission | 58 | 26 (44.8) | 29 | 5 (17.2) | 0.01 |
| Death | 69 | 7 (10.1) | 33 | 0 (0.0) | 0.01 |

*Except where indicated otherwise, data are the number (%).
†Administered biological medications included anakinra, tocilizumab, canakinumab, etanercept, abatacept, rituximab, alentuzumab.
‡The statistical comparison was made on the ensemble of triggering factors.
ICU, intensive care unit; JIA, juvenile idiopathic arthritis; MAS, macrophage activation syndrome.

most frequently assigned the highest score of 5, whereas platelet count was scored most commonly as 4; AST most frequently received scores of 3, 2 and 1. Haemoglobin was the least frequently selected test, whereas D-dimer had the lowest global score.

### Final rank of laboratory tests at the consensus conference

After three voting sessions, the experts selected and ranked 9 of the 11 laboratory parameters that were examined (table 4). Ferritin was the parameter that received the highest score, followed by platelet count, AST, fibrinogen, neutrophil count, WCC count, lactate dehydrogenase (LDH), ESR and D-dimer. Haemoglobin and triglycerides, among the five most important laboratory tests, were not selected by any expert.

## DISCUSSION

Using a data-driven and consensus formation approach, we identified the laboratory tests in which early change is most valuable for the timely diagnosis of MAS in the setting of sJIA. Platelet count, ferritin and AST were

**Table 2** Comparison of dynamics of laboratory tests over the course of MAS between patients classified as MAS or non-MAS by the expert panel*

| | Patients classified as MAS (n=70) | | | | | Patients classified as non-MAS (n=33) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Laboratory test | n | Value at last visit before MAS onset | Value at MAS onset | Absolute change | Percentage change | n | Value at last visit before MAS onset | Value at MAS onset | Absolute change | Percentage change | p Value† | p Value‡ |
| Hb, g/dL | 70 | 11.2 | 9.9 | −1.1 | −10 | 33 | 11.5 | 10.8 | −0.5 | −5 | 0.070 | 0.030 |
| WCC, $\times 10^9$/L | 70 | 15.0 | 7.4 | −5.5 | −50 | 33 | 10.8 | 12.0 | 1.6 | 11 | <0.0001 | <0.0001 |
| N count, $\times 10^9$/L | 57 | 10.6 | 4.7 | −5.5 | −64 | 31 | 6.9 | 7.6 | 1.4 | 18 | <0.0001 | <0.0001 |
| PLT, $\times 10^9$/L | 69 | 337 | 111 | −209 | −63 | 33 | 381 | 314 | −62 | −15 | <0.0001 | <0.0001 |
| ESR, mm/h | 62 | 59 | 26 | −21 | −39 | 26 | 33 | 61 | 8 | 59 | <0.0001 | <0.0001 |
| AST, U/L | 67 | 33 | 176 | 133 | 379 | 32 | 33 | 74 | 17 | 49 | 0.0002 | 0.0003 |
| LDH, U/L | 46 | 501 | 1735 | 1158 | 216 | 24 | 592 | 721 | 191 | 25 | <0.0001 | <0.0001 |
| Triglycerides, mg/dL | 42 | 120 | 257 | 116 | 111 | 13 | 93 | 126 | 32 | 33 | 0.006 | 0.020 |
| Fibrinogen, mg/dL | 44 | 456 | 201 | −183 | −47 | 20 | 510 | 454 | −71 | −22 | 0.010 | 0.010 |
| Ferritin, ng/mL | 60 | 875 | 10 516 | 7376 | 819 | 27 | 200 | 1183 | 548 | 282 | <0.0001 | 0.03 |
| D-dimer, ng/mL | 23 | 1705 | 4620 | 2000 | 244 | 13 | 576 | 1237 | 300 | 100 | 0.09 | 0.19 |

*Values are the median (the IQRs can be provided on request to the authors). Absolute and percentage changes are the median values of the changes recorded in each individual patient.
†The p value refers to the comparison between absolute changes.
‡The p value refers to the comparison between percentage changes.
AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; N, neutrophils; PLT, platelets; WCC, white cell count.

**Table 3** Number and percentage of instances in which each laboratory test was selected by the experts and number of instances in which each individual score was assigned by the experts to each laboratory test during web consensus procedures

| Laboratory test | N selected/n available (%) | Number of attributions of individual scores* | | | | | Sum of scores |
|---|---|---|---|---|---|---|---|
| | | 5 | 4 | 3 | 2 | 1 | |
| Platelet count | 1635/1821 (90) | 679 | 609 | 238 | 78 | 31 | 6732 |
| Ferritin | 1363/1580 (86) | 818 | 240 | 153 | 93 | 59 | 5754 |
| Aspartate aminotransferase | 1247/1767 (71) | 30 | 118 | 322 | 477 | 300 | 2842 |
| Fibrinogen | 689/1164 (59) | 27 | 143 | 169 | 184 | 166 | 1748 |
| Neutrophil count | 707/1502 (47) | 74 | 221 | 168 | 132 | 112 | 2134 |
| Lactate dehydrogenase | 529/1212 (44) | 2 | 43 | 104 | 171 | 209 | 1045 |
| White cell count | 769/1847 (42) | 67 | 199 | 207 | 128 | 168 | 2176 |
| D-dimer | 254/606 (42) | 1 | 16 | 53 | 78 | 106 | 490 |
| Triglycerides | 429/1110 (39) | 0 | 6 | 50 | 130 | 243 | 677 |
| Erythrocyte sedimentation rate | 555/1632 (34) | 13 | 78 | 150 | 149 | 165 | 1290 |
| Haemoglobin | 398/1847 (22) | 4 | 42 | 101 | 95 | 156 | 837 |

*The score 5 was assigned to the most important laboratory test, whereas the score 1 was assigned to the least important.

agreed on by a panel of experts, as being most important after the web-based evaluation of a large sample of sJIA with MAS patient profiles and face-to-face discussion, and voting at the consensus conference.

In a previous analysis of the dynamics of laboratory values over the course of MAS, Minoia *et al*[12] found that the three selected parameters, together with triglycerides and LDH, followed the expected trend of change in >90% of patients. Furthermore, platelet count, ferritin and liver transaminases were among the five tests that showed a percentage change of >50% between pre-MAS visit and MAS onset. Of the five laboratory biomarkers (which did not include ferritin and liver transaminases) evaluated by Lehmberg *et al*,[23] platelet count displayed the largest decline between the measurements made before the diagnosis of MAS and at the time of diagnosis of MAS. Altogether, these findings are in keeping with the experts' choices.

Haemoglobin and triglycerides were the sole categories, among the five most important laboratory tests, that were never selected by the experts at the consensus conference. The less relevance given to haemoglobin may be explained by the notion that children with active sJIA often have marked anaemia as part of the underlying inflammatory process.[24 25] Thus, the experts might have perceived that when MAS develops there can frequently be limited room for a further decrease in haemoglobin. Note that, in the aforementioned Minoia *et al*[12] evaluation of the dynamics of laboratory values over time, haemoglobin demonstrated a small median percentage change (−8.8%).

**Table 4** Scores assigned to laboratory tests at the consensus conference

| Laboratory test | Score |
|---|---|
| Ferritin | 109 |
| Platelet count | 105 |
| Aspartate aminotransferase | 58 |
| Fibrinogen | 32 |
| Neutrophil count | 20 |
| Lactate dehydrogenase | 15 |
| White cell count | 13 |
| Erythrocyte sedimentation rate | 5 |
| D-dimer | 3 |
| Haemoglobin | – |
| Triglycerides | – |

The lack of choice of triglycerides is somewhat surprising, however. An increased triglyceride level is one of the laboratory abnormalities included in the new classification criteria for MAS complicating sJIA.[16] In addition, in the Minoia *et al*[12] analysis, triglycerides were among the laboratory tests that followed the expected trend of change in more than 90% of patients, and displayed a percentage change of >50% between pre-MAS visit and MAS onset. It can be hypothesised that the experts felt that the variation in triglyceride level lags behind that of other laboratory parameters or that there could be a small dynamic change in level. Nevertheless, the time course of triglyceridaemia during MAS should be further explored in a prospective study.

Several episodes of MAS in patients with sJIA under treatment with the cytokine blockers canakinumab and tocilizumab have been recently observed in randomised controlled clinical trials and in postmarketing experience.[26–29] Because these agents inhibit the biological effects of IL-1 and IL-6, respectively, which are among the pro-inflammatory cytokines involved in the physiopathology of MAS,[1 30] it is conceivable that their administration may modify the clinical and biological presentation of MAS. Clinical symptoms of patients with sJIA-associated MAS receiving tocilizumab were found to be milder than those of patients not receiving this medication.[31] However, more data from the real world of

clinical practice are needed to establish whether the change in laboratory parameters over the course of MAS occurring during treatment with IL-1 and IL-6 inhibitors is more subtle than that in other instances of the syndrome.

Our study should be interpreted in the light of some potential caveats. All the study cases were defined as MAS based on clinician expert opinion. However, because all patient profiles were reviewed by the experts and the diagnosis of MAS or non-MAS was confirmed only when a high level of consensus was reached, the impact of this potential limitation was likely minimised. Some important diagnostic parameters of MAS, such as sCD25 and sCD163 levels, and natural killer cell activity, could not be assessed owing to their unavailability in all patient samples. However, in most paediatric rheumatology centres, these biomarkers are neither routinely assessed nor timely. Notably, other ongoing efforts, including the study of patient cytokine profiles, may help in the perspective to distinguish MAS from active sJIA.[32]

We should also acknowledge that, because serial values of laboratory tests were available for patients with MAS, but not for control groups of patients with potentially confusable conditions, we could not establish the threshold level of change in each test that had the best sensitivity and specificity for the detection of MAS. Furthermore, as we did not ask the investigators who entered their patients' information to include the date of the last visit before the onset of MAS, we were unable to standardise the time lag between the visits before onset of MAS and at onset of MAS. These limitations precluded the incorporation of the change in laboratory values over time in the new classification criteria for MAS complicating sJIA.[16]

In summary, we identified the laboratory tests (platelet count, and serum ferritin and AST levels) in which changes over time are most valuable for the timely diagnosis of MAS occurring in the context of sJIA. The dynamics of laboratory values during the course of MAS should be further scrutinised prospectively at standardised time points and with the inclusion of appropriate groups of control patients in order to establish the optimal cut-off values for their early variation.

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**Acknowledgements** The authors thank the PRINTO employees, Simona Angioloni, Chiara Pallotti, Michele Pesce, Mariangela Rinaldi and Luca Villa, for their technical assistance and for providing the technical and secretarial assistance in the organisation of the consensus conference. The authors also thank the following physicians who contributed to the study by including their patients' data: Mario Abinun, MD (Newcastle, UK); Amita Aggarwal, MD (Lucknow, India); Jonathan Akikusa, MD (Melbourne, Australia); Sulaiman M Al-Mayouf, MD (Riyadh, Saudi Arabia); Maria Alessio, MD (Napoli, Italy); Jordi Anton, MD (Barcelona, Spain); Maria Teresa Apaz, MD (Cordoba, Argentina); Itziar Astigarraga, MD (Bilbao, Spain); Nuray A Ayaz, MD (Istanbul, Turkey); Patrizia Barone, MD (Catania, Italy); Blanca Bica, MD (Rio de Janeiro, Brazil); Isabel Bolt, MD (Berne, Switzerland); Luciana Breda, MD (Chieti, Italy); Vyacheslav Chasnyk, MD (Saint-Petersburg, Russian Federation); Rolando Cimaz, MD (Firenze, Italy); Fabrizia Corona, MD (Milan, Italy); Ruben Cuttica, MD (Buenos Aires, Argentina); Gianfranco D'Angelo, MD (Ancona, Italy); Zane Davidsone, MD (Riga, Latvia); Carmen De Cunto, MD (Buenos Aires, Argentina); Jaime De Inocencio, MD (Madrid, Spain); Eli Eisenstein, MD (Jerusalem, Israel); Sandra Enciso, MD (Mexico City, Mexico); Graciela Espada, MD (Buenos Aires, Argentina); Michel Fischbach, MD (Muenster, Germany); Michael Frosch, MD (Muenster, Germany); Romina Gallizzi, MD (Messina, Italy); Maria Luz Gamir, MD (Madrid, Spain); Yi-Jin Gao, MD (Shanghai, China); Thomas Griffin, MD (Charlotte, Carolina, USA); Soad Hashad, MD (Tripoli, Libya); Teresa Hennon, MD (Buffalo, New York, USA); Gerd Horneff, MD (Sankt Augustin, Germany); Zeng Huasong, MD (Guangzhou, China); Adam Huber, MD (Halifax, Canada); Norman Ilowite, MD (New York, New York, USA); Antonella Insalaco, MD (Roma, Italy); Maka Ioseliani, MD (Tbilisi, Georgia); Marijia Jelusic-Drazic, MD (Zagreb, Croatia); Michael Jeng, MD (Stanford, California, USA); Agneza Kapovic, MD (Zagreb, Croatia); Ozgur Kasapcopur, MD (Istanbul, Turkey); Toshiyuki Kitoh, MD (Nagakute, Japan); Isabelle Kone-Paut, MD (Paris, France); Sheila Knupp Feitosa de Oliveira, MD (Rio de Janeiro, Brazil); Bianca Lattanzi, MD (Ancona, Italy); Loredana Lepore, MD (Trieste, Italy); Caifeng Li, MD (Beijing, China); Jeffrey M Lipton, MD (New York, New York, USA); Silvia Magni-Manzoni, MD (Rome, Italy); Despoina Maritsi, MD (Athens, Greece); Deborah McCurdy, MD (Orange, California, USA); Rosa Merino, MD (Madrid, Spain); Velma Mulaosmanovic, MD (Sarajevo, Bosnia and Herzegovina); Susan Nielsen, MD (Copenhagen, Denmark); Priyankar Pal, MD (Kolkata, India); Sampath Prahalad, MD (Atlanta, Georgia, USA); Donato Rigante, MD (Roma, Italy); Ingrida Rumba-Rozenfelde, MD (Riga, Latvia); Claudia Saad Magalhaes, MD (Botucatu, Brazil); Helga Sanner, MD (Oslo, Norway); Sujata Sawhney, MD (New Delhi, India); Wafaa M Sewairi, MD (Riyadh, Saudi Arabia); Bita Shakoory, MD (Philadelphia, Pennsylvania, USA); Susan Shenoi, MD (Seattle, USA); Artur Silva Clovis, MD (Sao Paulo, Brazil); Valda Stanevicha, MD (Riga, Latvia); Kimo C Stine, MD (Little Rock, Arkansas, USA); Gordana Susic, MD (Belgrade, Serbia); Flavio Sztajnbok, MD (Rio de Janeiro, Brazil); Syuji Takei, MD (Kagoshima City, Japan); Hasan Tezer, MD (Ankara, Turkey); Ralf Trauzeddel, MD (Berlin, Germany); Elena Tsitsami, MD (Athens, Greece); Erbil Unsal, MD (Izmir, Turkey); Olga Vougiouka, MD (Athens, Greece); Lehn K Weaver, MD (Philadelphia, Pennsylvania, USA); Jennifer Weiss, MD (Hackensack, New Jersey, USA); Sheila Weitzman, MD (Toronto, Canada); Mabruka Zletni, MD (Tripoli, Libya).

**Funding** This work is supported by the American College of Rheumatology (ACR), the European League Against Rheumatism (EULAR) and the Pediatric Rheumatology International Trials Organization.

**Competing interests** None declared.

**Ethics approval** Ethics committee at each participating centre.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** No additional unpublished data are available.



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